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DMSO enhances electrospray response, boosting sensitivity of proteomic experiments

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We report that low percentages of dimethylsulfoxide (DMSO) in liquid chromatography solvents lead to a strong enhancement of electrospray ionization of peptides, improving the sensitivity of protein identification in bottom-up proteomics by up to tenfold. The method can be easily implemented on any LC-MS/MS system without modification to hardware or software and at no additional cost.

The *de facto* standard analytical platform for the identification and quantification of peptides and proteins in current proteomics is reversed-phase liquid chromatography coupled to electrospray ionization tandem mass spectrometry¹ (LC-MS/MS). The field is still driven by improvements in technology, which enables the analysis of ever more complex proteomes (5–10,000 proteins, more than six orders of magnitude of expression range) at ever decreasing sample quantities (low-microgram scale)^{2–4}. Despite the high sensitivity of modern mass spectrometers, the efficiency of the overall LC-MS/MS process is still rather low; much of the available peptide sample does not actually enter the mass spectrometer as gas-phase ions⁵. Numerous efforts have been made to improve this step—including the development of ion funnels or other ion-focusing devices⁶ to improve capture of the ions generated by electrospray, as well as the more experimental use of multiple electrospray emitters⁷ to enhance electrospray ionization (ESI) efficiency itself.

A recent study⁸ investigated the effects of the supercharging reagents DMSO and *m*-nitrobenzyl alcohol (*m*-NBA)⁹ on the identification of peptides derived from trypsin, elastase or pepsin digestion of a five-protein mixture using LC-MS/MS. This revealed that the addition of 5% DMSO to the LC solvents increased

the number of identified peptides by 10–25%. The authors concluded that this gain resulted from DMSO causing charge-state coalescence of peptide precursor ions resulting in simpler precursor ion spectra and improved MS/MS data supporting peptide identification by database searching. This observation prompted us to more systematically investigate the effects of DMSO on the performance of peptide and protein identification by LC-MS/MS.

Using a complex tryptic digest of HeLa cells and an Orbitrap Elite mass spectrometer, we found that the addition of 5% DMSO to the LC solvents increased the mass spectrometric signal intensity of peptides by a median factor of three (calculated on the basis of extracted precursor-ion chromatograms of all identified peptides) (Fig. 1a,b). The increase in signal concomitantly led to a decrease of injection times for full-scan mass spectra also by a median factor of three (Fig. 1c), demonstrating that the gain in signal intensity is due to more ions entering the mass spectrometer (that is, improved ESI efficiency) rather than merely an improved signal-to-noise ratio. Ion-injection times were also considerably lower for precursor ion selection for MS/MS (Fig. 1c), leading to a substantial increase in the number of peptides and proteins identified and quantified (Fig. 1d and Supplementary Fig. 1). Low-abundance peptides benefited more from DMSO addition than high-abundance peptides (median of 6-fold for the lowest-intensity quintile versus 3.3-fold for the highest-intensity quintile, Supplementary Fig. 2), suggesting a rationale for the disproportionately improved rate of peptide and protein identification without a corresponding increase in the acquisition rate for tandem mass spectra. The addition of DMSO is also particularly advantageous in the analysis of small quantities of protein digests, for which the detection limit for peptide and protein identification improved up to tenfold (Fig. 1e). The performance increase also extended, albeit to a lesser extent, to analyses of post-translational modifications such as phosphorylation and acetylation (Supplementary Fig. 3), and of low and medium complex peptide mixtures such as affinity purifications¹⁰ (Supplementary Fig. 4). A practical consequence of the use of DMSO is that it allows a reduction of the analysis time while essentially generating the same information for a given sample.

We analyzed the MS data for the charge-state coalescence phenomenon observed previously⁸ and found that this accounts for merely 10–20% of the signal improvement (Supplementary Figs. 5–7), suggesting that the ionization process itself is responsible for the overall gain in sensitivity. Our analysis also suggests that DMSO generally induces charge-state reduction (Supplementary Fig. 6) because whereas the population of doubly charged precursor ions increased substantially in the

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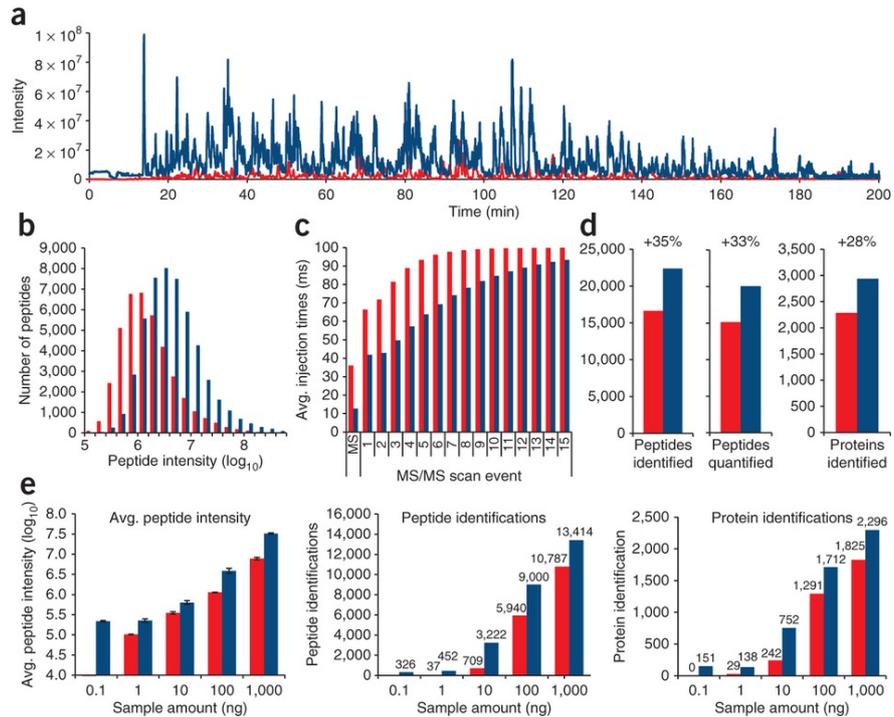
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Figure 1 | DMSO improves the performance of LC-MS/MS–based bottom-up proteomics. **(a)** Base peak chromatogram of a tryptic digest of HeLa cells in the absence (red)

or presence (blue) of 5% DMSO in reversed-phase LC solvents. **(b)** Intensity distribution (log₁₀) of number of identified HeLa peptides in the absence (red) or presence (blue) of 5% DMSO. **(c)** Average ion-injection time for full-scan mass spectra (MS) up to 15 consecutive tandem mass spectra (MS/MS scan event). **(d)** Numbers of peptides and proteins identified and/or quantified in 1 μg tryptic HeLa digest (total non redundant peptide and protein identifications of a triplicate analysis with (blue) or without (red) 5% DMSO, 210 min LC-MS/MS gradient). **(e)** Serial dilution of the HeLa digest



(triplicate analysis, 60-min LC-MS/MS gradient) showing the effect of DMSO on average peptide intensity (error bars, \pm s.d.), peptide identifications and protein identifications (total non redundant peptide and protein identifications of the triplicate analysis).

presence of DMSO, higher charge states of peptides were diminished. This can be explained by the relatively high gas-phase proton affinity of DMSO¹¹ (884 kJ/mol), which may lead to proton stripping from tryptic peptides of higher charge states for which a proton is not localized at the N terminus or the C-terminal arginine and lysine residues (proton affinity of glycine-glycine, 882 kJ/mol; glycine-alanine, 888 kJ/mol; arginine, 1,051 kJ/mol; lysine, 996 kJ/mol)¹¹. The shift toward doubly charged precursor ions is advantageous both in terms of sensitivity, as most of the ionization is concentrated in one charge state, and for peptide identification by database searching, because current algorithms perform particularly well on singly charged fragment ions. Collectively, these differences lead to considerably higher peptide identification scores in the presence of DMSO (median score increase = 0.5 log₁₀, equivalent to a three-fold improvement in probability, Supplementary Fig. 1).

DMSO is a polar aprotic solvent with an elution strength similar to that of acetonitrile. Therefore, addition of DMSO to LC solvents requires adaptation of the gradient-elution profile to avoid the loss of hydrophilic peptides (Fig. 2a and Supplementary Fig. 8). In our LC setup, we also found it necessary to omit DMSO from the solvent in which peptides are loaded onto the LC trap column, as its addition led to a marked overall loss of peptides. These parameters may vary depending on the

setup and stationary phase used and should, therefore, be adjusted on a case-by-case basis. Alternatively, DMSO can be added after the column to avoid such losses. We also found that the presence of DMSO in LC solvents greatly reduces column carryover effects. The resolution of peptide separations was essentially unchanged in the presence of DMSO; for a 210-min separation the LC peaks were only 1.9 s broader with DMSO (median) than without (Fig. 2b), and the order in which peptides eluted over time was also practically the same with and without DMSO (Fig. 2c). The detection of hydrophilic peptides, notably acidic and methionine-oxidized peptides, was particularly improved in the presence of DMSO (Supplementary Figs. 3 and 9). Another marked effect of DMSO in LC solvents was the complete repression of the frequently observed spectral background that arises from the ionization of polysiloxane molecules from ambient air (Fig. 2d). This repression is likely to be due to the higher gas-phase proton affinity of DMSO (884 kJ/mol) compared to siloxanes (846 kJ/mol for hexamethyldisiloxane)¹¹, which might lead to the suppression of ionization or deprotonation of the polysiloxanes in the gas phase. As a consequence of this effect, the signal background featured DMSO-containing ions (Fig. 2d), including a species of the elemental composition [C₆H₁₀O₁₄S₃]⁺ at m/z 401.922718, which can be used as a stable lock

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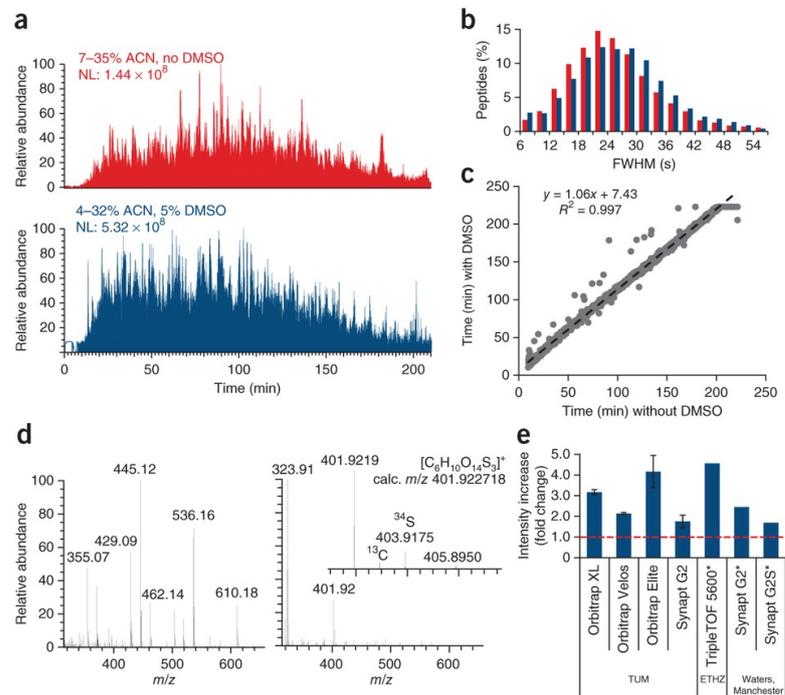
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Figure 2 | Impact of DMSO on the chromatographic behavior of peptides, the mass spectrometric signal background and the performance of different LC-MS/MS systems. **(a)** LC-MS/MS analysis (Orbitrap Elite) of a complex HeLa tryptic digest in the absence (top) and presence (bottom) of 5% DMSO. ACN, acetonitrile; NL, normalized intensity. **(b)** Analysis of the chromatographic peak widths (full width at half maximum (FWHM) intensity; 210 min total gradient time; Orbitrap Elite). **(c)** The elution times for peptides (>8,000) detected with and without DMSO correlate strictly linearly, with slope = 1. **(d)** The polysiloxane background (left) typically observed in LC-MS/MS experiments

is completely suppressed in the presence of DMSO (right). The signal at m/z 401.922718 can be used as a lock mass. **(e)** Results obtained on seven mass spectrometers in three laboratories with different ESI source configurations and LC stationary phases show an increase by a factor of 24 (summed peptide intensities for triplicate measurements at the Technical

University of Munich; error bars, \pm s.d. of fold change; asterisks indicate base peak intensities for single measurements taken in Zurich (ETHZ) or Manchester, UK (Waters, Manchester).



mass12 for dynamic recalibration of the mass spectrometer (Supplementary Fig. 10). To evaluate whether DMSO-mediated signal enhancement is a general phenomenon of peptide ESI, we implemented the method in three laboratories and on seven LC-MS/MS systems using different electrospray emitters, ESI source designs, front-end ion optics and LC column material. We observed a substantial (two- to fourfold) signal increase on all instruments (Fig. 2e and Supplementary Figs. 11–13).

As discussed above, the substantial increase in peptide signal intensity observed in the presence of DMSO cannot be explained by charge-state coalescence, improved LC resolution or background-ion suppression. Instead, our data point to more efficient production of ions during the electrospray process. Our working model on the mechanism by which this occurs is based on classical electrospray theory¹³ (Supplementary Note and Supplementary Fig. 14). Owing to the low surface tension of DMSO, we propose that even low percentages of DMSO lead to faster and, probably, more complete sequestration of single peptide molecules into charged nanoscale droplets (in situ nanoelectrospray), and that this in turn increases the likelihood of ionization. This mechanistic interpretation is supported by several lines of experimental evidence. First, we measured peptide intensities in the presence and absence of DMSO at varying LC flow rates (Supplementary Fig. 15). As expected, peptide intensities increased with decreasing

flow rate in the absence of DMSO. In contrast, decreasing flow rates in the presence of DMSO had no appreciable effect on peptide ionization efficiency. Second, the disproportional increase in the detection of hydrophilic and, particularly, acidic peptides under DMSO conditions (Supplementary Fig. 9) can be explained by the sequestration of single analyte molecules into single droplets, which removes the bias of ESI toward basic peptides. Third, even low concentrations of DMSO lead to the observed effect, as predicted by the model (Supplementary Fig. 16). Fourth, the dilution experiment of a complex digest (Fig. 1e) revealed a disproportional benefit of DMSO for low sample quantities, which can be explained only by improved peptide ionization. Fifth, the results obtained on the seven instruments used in this study suggest that the DMSO effect can be generalized (Supplementary Note).

The addition of low percentages of DMSO to LC solvents appears to be a generic approach to increasing the ESI response of peptides and, hence, the sensitivity of proteomic experiments. The performance gain observed is comparable to that of a new generation of mass spectrometric equipment, demonstrating the substantial analytical as well as economic value of the method. Furthermore, the method can be implemented without technical difficulty and at no cost on any of the thousands of LC-MS/MS systems in use worldwide for proteomic analysis. Although we have demonstrated the benefits of DMSO for peptide analysis only, it is entirely

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conceivable that small-molecule analysis by LC-MS/MS or other chromatographic techniques such as hydrophilic-interaction LC or normal-phase LC might also benefit. We therefore anticipate that the approach could have a substantial impact on the field of LC-MS/MS analysis in general and on proteomics in particular.

Methods

Methods and any associated references are available in the online version of the paper.

Accession codes.

The raw mass spectrometric data used in this study and the MaxQuant analysis files are available via ProteomicsDB (project number 4055) and via proteomeXchange (accession code PXD000254).

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Author Contributions

H.H., F.P., B.R., S.K.M., S.K., D.H., G.M. and S.L. performed experiments; M.W. made numerous intellectual contributions; B.K. conceptualized the work and H.H. and B.K. wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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Online Methods**Sample preparation.**

Mycoplasma-free HeLa S3 cervix carcinoma cells (DMSZ, Braunschweig, Germany) were cultured in DMEM with high glucose (PAA, Pasching, Austria) supplemented with 10% (v/v) FBS (FBS, PAA, Pasching, Austria) at 37 °C in humidified air and 10% CO₂. Cells were washed with PBS and lysed in 50 mM Tris-HCl (pH 7.5), 5% glycerol, 1.5 mM MgCl₂, 150 mM NaCl, 0.8% NP-40, 1 mM dithiothreitol and 25 mM NaF with freshly added protease inhibitors and phosphatase inhibitors (5× phosphatase inhibitor cocktail 1, Sigma-Aldrich, Munich, Germany; 5× phosphatase inhibitor cocktail 2, Sigma-Aldrich, Munich, Germany; 1 mM sodium orthovanadate and 20 nM Calyculin A, LC Laboratories, Woburn, MA, USA). Protein extracts were clarified by ultracentrifugation for 1 h at 145,000 × *g* at 4 °C, and protein concentration was determined by the Bradford method. Lysates were denatured in 8 M urea and 0.1 M Tris-HCl and subsequently diluted to 2 M urea, then protein was digested with trypsin (Promega) according to the filter-aided sample preparation (FASP) protocol¹⁴. After overnight digestion, peptides were eluted from the filters with 50 mM triethylammonium bicarbonate and purified on C₁₈ StageTips as described¹⁵. Affinity purification of kinases from human cell lines using the Kinobead method was performed as described¹⁰. Immobilized metal affinity chromatography (IMAC) enrichment of phosphopeptides from human cell lines was performed as described¹⁶.

LC-MS/MS measurements.

LC-MS/MS was performed by coupling an Eksigent NanoLC-Ultra 1D+ (Eksigent, Dublin, CA) to an Orbitrap Elite instrument (Thermo Scientific, Bremen, Germany). One microgram of the HeLa digest was delivered to a trap column (100 μm × 2 cm, packed in-house with Reprosil-Pur C₁₈-AQ 5 μm resin, Dr. Maisch, Ammerbuch, Germany) at a flow rate of 5 μL/min in 100% solvent A (0.1% formic acid (FA), in HPLC grade water). After 10 min of loading and washing, peptides were transferred to an analytical column (75 μm × 40 cm, packed in-house with Reprosil-Gold C₁₈, 3 μm resin, Dr. Maisch, Ammerbuch, Germany) and separated at a flow rate of 300 nL/min using a 210-min gradient. DMSO was obtained from Sigma-Aldrich (article no. 41647). Measurements without DMSO used a gradient from 7% to 35% solvent B (solvent A, 0.1% FA in HPLC-grade water; solvent B, 0.1% FA in acetonitrile). Measurements with DMSO used a gradient from 4% to 32% solvent D (solvent C, 0.1% FA and 5% DMSO in HPLC-grade water; solvent D, 0.1% FA and 5% DMSO in acetonitrile). The HeLa digest dilution series (0.11,000 ng digest on column) was analyzed in triplicates using a 60-min gradient and the same solvents described above.

automatically switching between MS and MS₂. Full-scan MS spectra (m/z 300 – 1300) were acquired in the Orbitrap at 30,000 (m/z 400) resolution using an automatic gain control (AGC) target value of 1 × 10⁶ charges. Tandem mass spectra of up to 15 precursors were generated in the multipole collision cells by using higher energy collisional dissociation (HCD) (AGC target value 2 × 10⁴, normalized collision energy of 30%) and analyzed in the Orbitrap at a resolution of 15,000. Precursor ion isolation width was set to 2.0 Th, the maximum injection time for MS/MS was 100 ms and dynamic exclusion was set to 30 s.

Measurements using an LTQ-Orbitrap XL or an Orbitrap Velos used the same LC system, column and gradient described above and similar data-acquisition parameters. The main differences were that the Orbitrap Velos used HCD of the top ten precursor ions at an AGC target value of 4 × 10⁴ and Orbitrap readout at a resolution of 7,500. Data acquired on the LTQ-Orbitrap XL used CID fragmentation of the top eight precursor ions and an AGC target value of 5 × 10³. Data acquired on the Waters Synapt G2 used the MSE method cycling between high and low collision energy every 0.7 s. Peptides were separated using the gradients described above by a nanoACQUITY LC system using a 15 cm × 75 μm (1.7 μm BEH130 material) column.

Peptide and protein identification and quantification. Raw MS data were processed by MaxQuant (version 1.3.0.3) for peak detection and quantification¹⁷. MS/MS spectra were searched against the IPI human database (version 3.68; 87,061 sequences, supplemented with 262 common contaminants) using the Andromeda search engine¹⁸ with the following search parameters: full tryptic specificity, up to two missed cleavage sites, carbamidomethylation of cysteine residues set as a fixed modification, and N-terminal protein acetylation and methionine oxidation as variable modifications. Mass spectra were recalibrated within MaxQuant (first search 20 p.p.m. precursor tolerance) and subsequently re-searched with a mass tolerance of 6 p.p.m. Fragment ion mass tolerance was set to 20 p.p.m. Search results were filtered to a maximum false discovery rate (FDR) of 0.01 for proteins and peptides and a minimum peptide length of at least 6 aa was required.

Chromatographic resolution (peak width, retention time) was assessed using a custom Java script, which automatically reconstructs elution profiles of identified peptides and approximates the full width at half maximum using a Gaussian fit.

The eluent was sprayed via stainless steel emitters (Proxeon) at a spray voltage of 2.2 kV and a heated capillary temperature of 275 °C. The Orbitrap Elite instrument was operated in data-dependent mode,